**TITLE:** THE USE OF *GALLERIA MELLONELLA* INFECTION MODEL TO ASSESS THE *IN VIVO* VIRULENCE OF ENTEROAGGREGATIVE *E. COLI*

**AUTHORS:** GUERRIERI, C.G.; PEREIRA, M.F.; GALDINO, A.C.M.; SANTOS, A.L.S.; SCHUENCK, R.P.; SPANO, L.C.

**INSTITUTION:** UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO (AV. MARECHAL CAMPOS 1468, CEP 29043-900 VITÓRIA - ES, BRAZIL)

**ABSTRACT:**
Enteroaggregative *Escherichia coli* (EAEC), classified as typical (tEAEC) and atypical (aEAEC) according to the presence of the *AggR* regulon, is considered an emerging pathogen and has been highlighted as a cause of acute and persistent diarrhea in individuals of all ages in different regions of the world. Several studies suggest that tEAEC are more virulent and therefore more likely to cause diarrhea than aEAEC. Considering that *Galleria mellonella* larvae have been used as an alternative model for the study of the virulence of several human pathogens, we proposed to analyze if this model would reveal difference in the pathogenicity between tEAEC and aEAEC, carrying different phenotypic and genotypic characteristics of virulence. We selected ten EAEC strains (4 tEAEC and 6 aEAEC), previously characterized for the main virulence genes and DNA fragmentation with enzyme restriction followed by pulsed-field gel electrophoresis, in addition to the prototype strain EAEC042 and *E. coli* HB101. All strains had different PFGE pattern and profile of virulence genes. We analyzed: (i) phenotypic characteristics of virulence such as the production of hydrolytic enzymes (phytase, phospholipase, protease, esterase, hemolysin) and siderophores in agar and biofilm stained with crystal violet; (ii) pathogenicity in the *G. mellonella* model. The virulence assay of the strains was performed by inoculating 10 µL of bacterial inoculum into the haemocoel, at concentrations ranging from $10^4$ to $10^7$ UFC/larvae (20 larvae/concentration), and incubation at 37 °C. Mortality of the larvae was followed by 96h. Control was constituted by larvae inoculated with sterile PBS. We observed: (i) production of enzyme phytase and siderophores by all strains, only one hemolysin producer and all biofilm producers; (ii) that EAEC is able to kill the larvae in a concentration-dependent manner; (iii) that aEAEC strains presented greater virulence in the *G. mellonella* model than the tEAEC; (iv) that there was no relationship between the number or repertoire of virulence genes and the genetic background with larval death. These results suggest that virulence factors not yet identified could justify the death of the larvae in aEAEC strains or that *G. mellonella* would not be an adequate model to infer the difference in virulence of tEAEC and aEAEC.

**Keywords:** *EAEC, Galleria mellonella, Infection model, Virulence assay.*

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